

FIGURE 1--Replacement of ERP with small molecule

Primary screen

“peptide displacement assay”



Secondary screen

“functional assays”

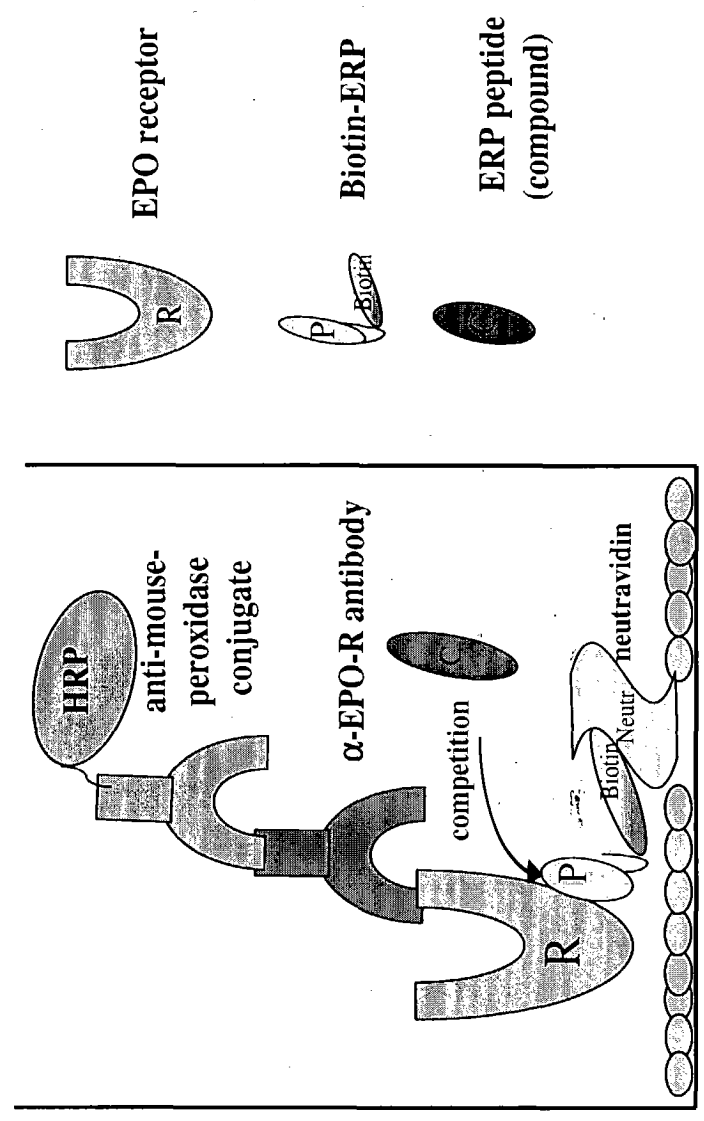


FIGURE 2--Role of small molecule in activation of EPO-R signaling pathway

Study of small molecule effects and potential applications

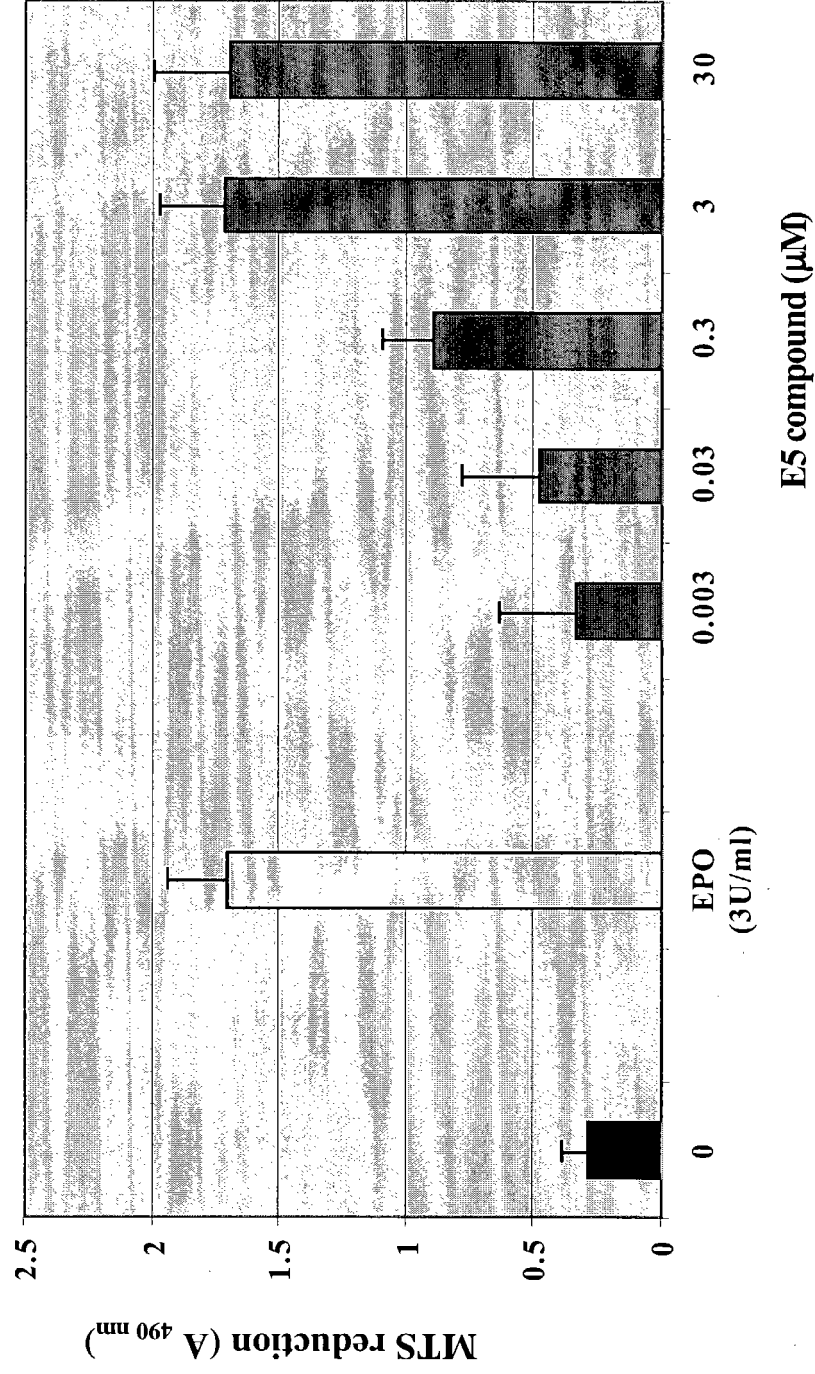
In vitro studies

- Erythroid system
 - Activation of proliferative and anti-apoptotic pathway(s)
 - Colony formation in fetal liver cells
 - CFU-e/BFU-e formation in bone marrow (mouse and human)
- CNS system
 - Activation of anti-apoptotic pathway
 - Survival of neural-like cells upon serum withdrawal

In vivo studies

- Effect in animals with carboplatin induced anemia; given I.p. and orally
- Reticulocytes levels in normal animals

FIGURE 3--Proliferative effect of E5 compound in
TF-1 cells

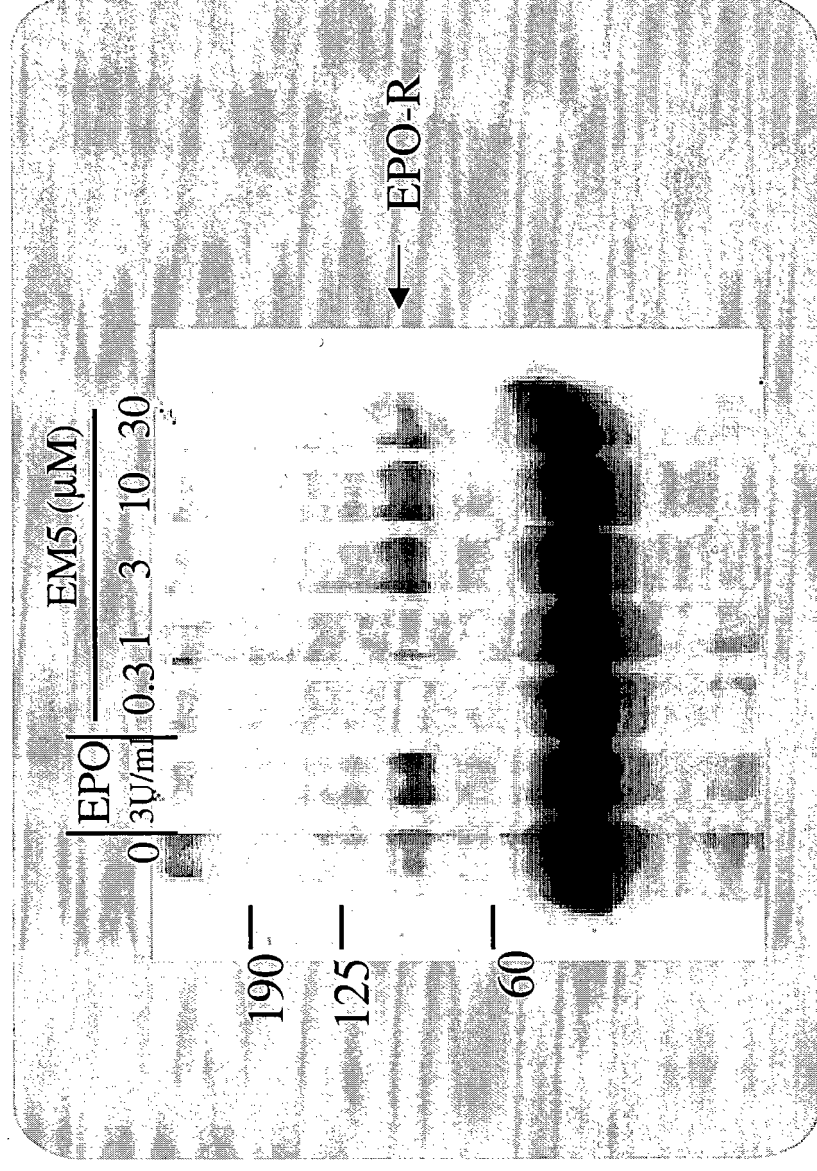


* Same proliferative effect is observed with UT-7 cells

* No effect in FDCP1 cells

FIGURE 4--Activation of signaling cascade through EPO-R by small molecule

Small molecules bind to and activate/phosphorylate EPO-R (UT-7 cells)



IP: α -EPO-R Ab (Upstate technology)

WB: α -PY Ab (Upstate technology)

FIGURE 5--Small molecules promote colony
formation in the presence of SCF
Fetal liver cells (day 3)

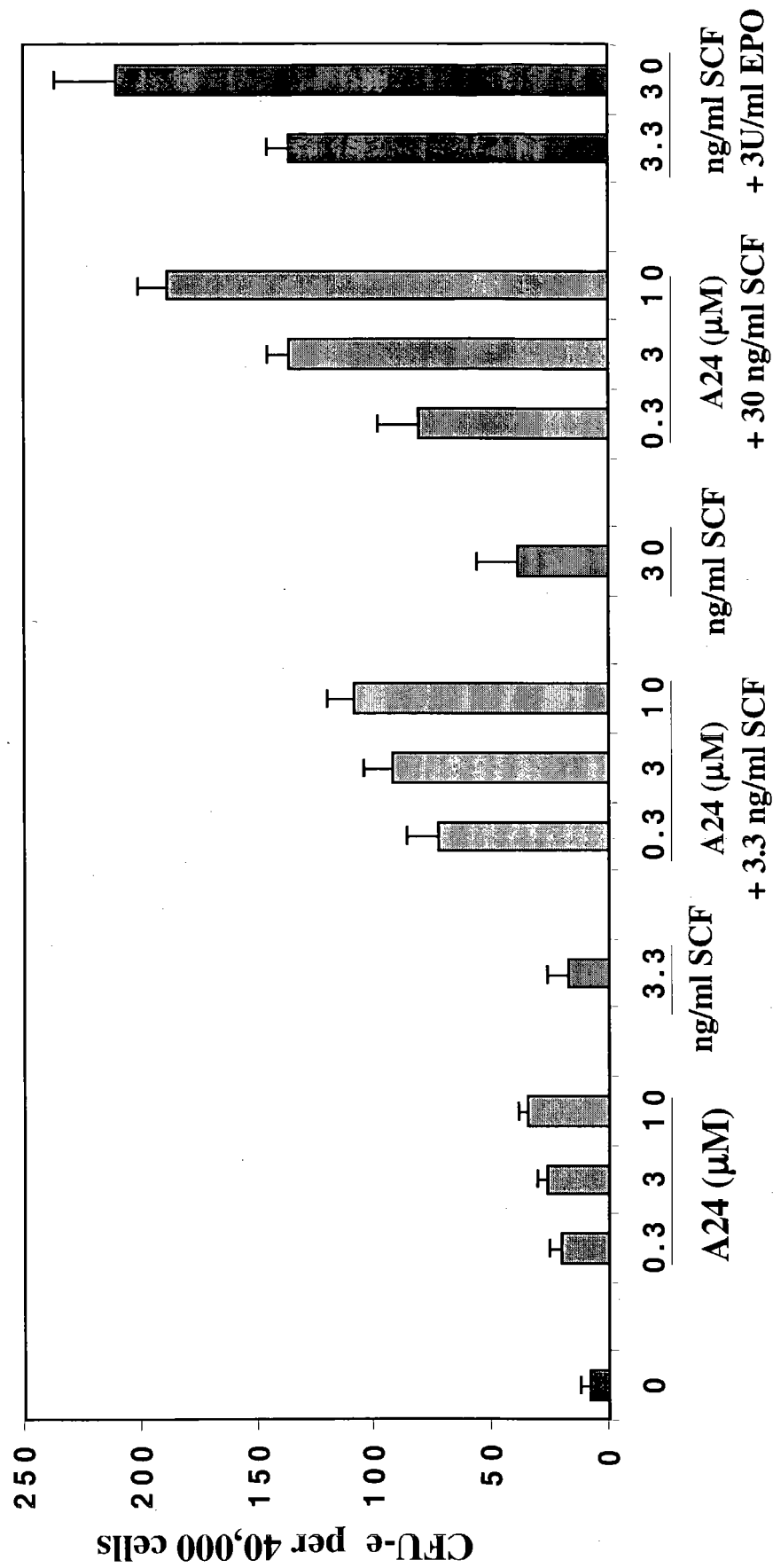


FIGURE 6--Effect of EPO-like small molecule on
erythroid colony formation in methylcellulose
Human bone marrow

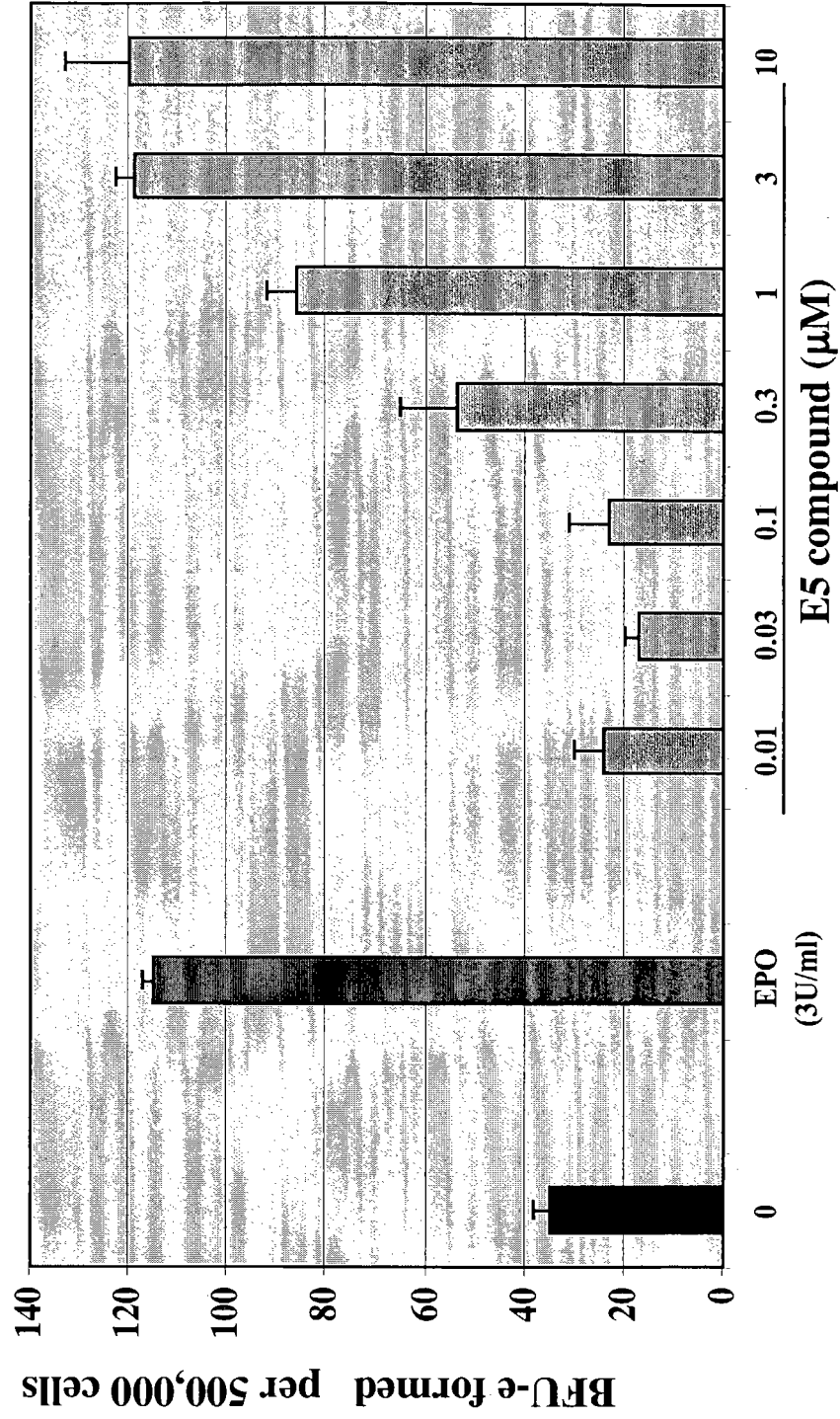
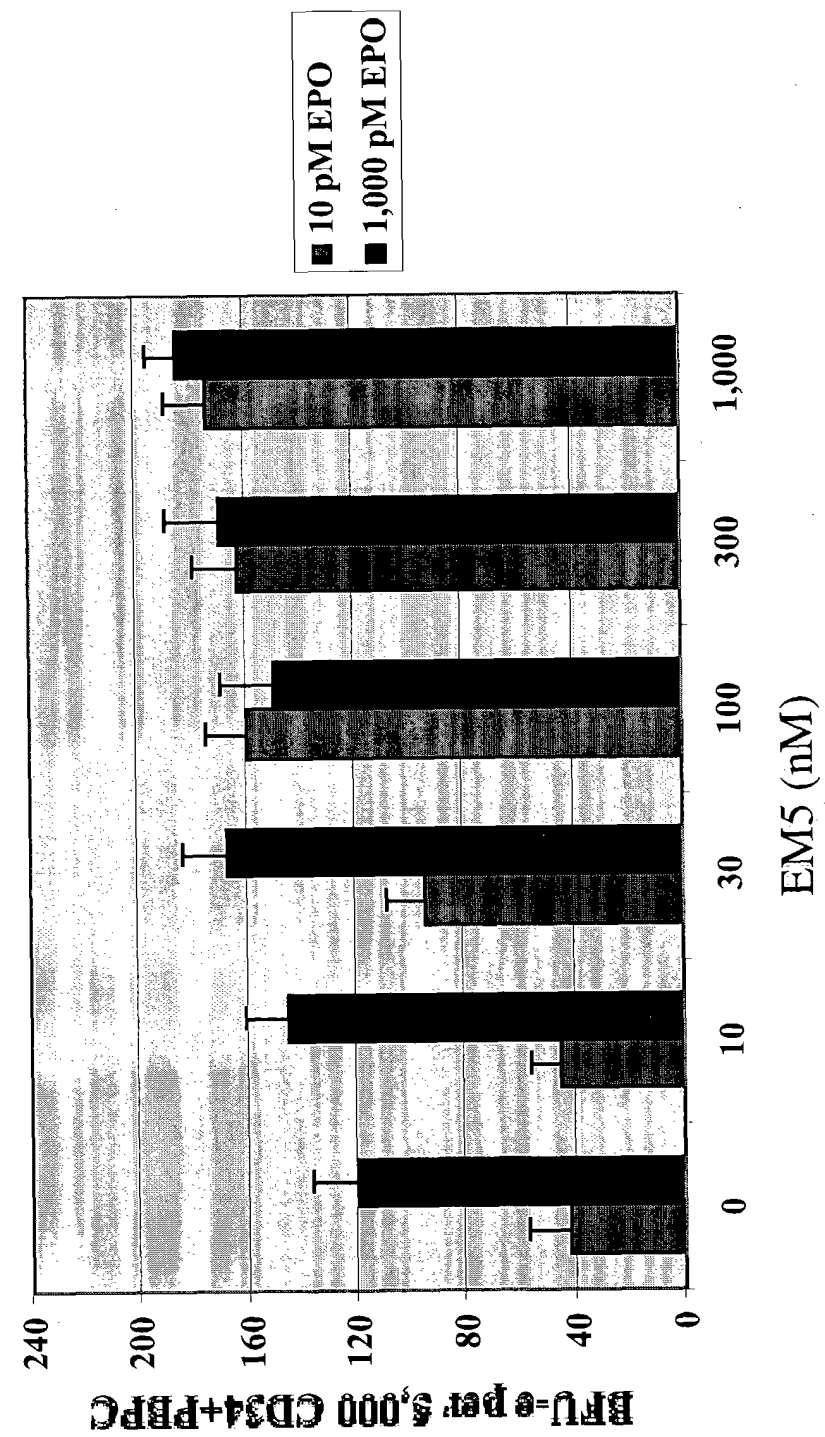


FIGURE 7-- Synergy between EM5 small molecule
and EPO on erythroid colony formation
Human bone marrow



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FIGURE 8 -- Synergy between E5A24 small molecule and EPO on erythroid colony formation
Human bone marrow (day 14)

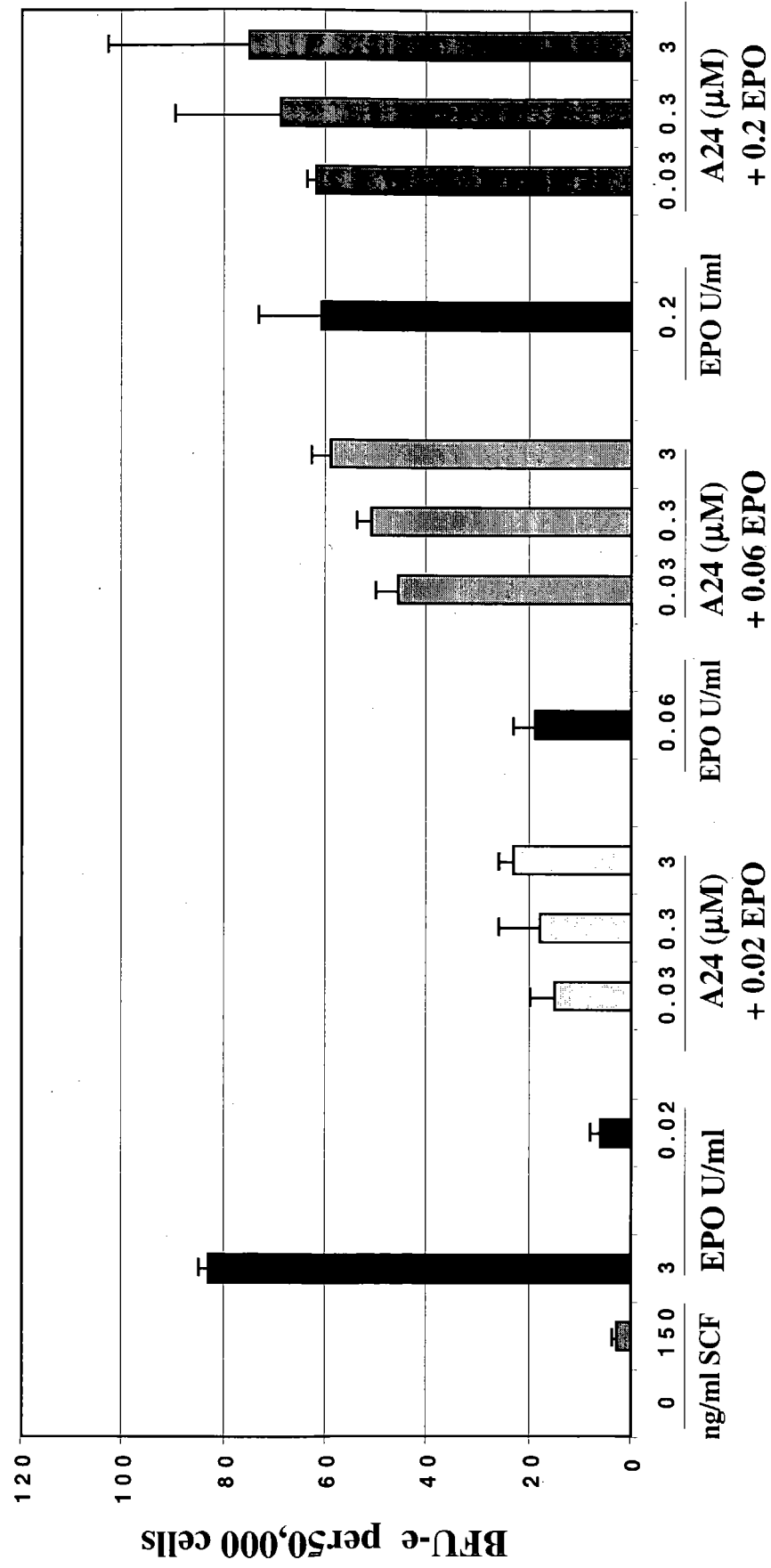


FIGURE 9

EFFECT OF EPO-LIKE
ACTIVITY, SMALL
MOLECULE - ON
HEMATOCRIT IN 8
WEEK OLD C57BL
MALE MICE

Dose-response

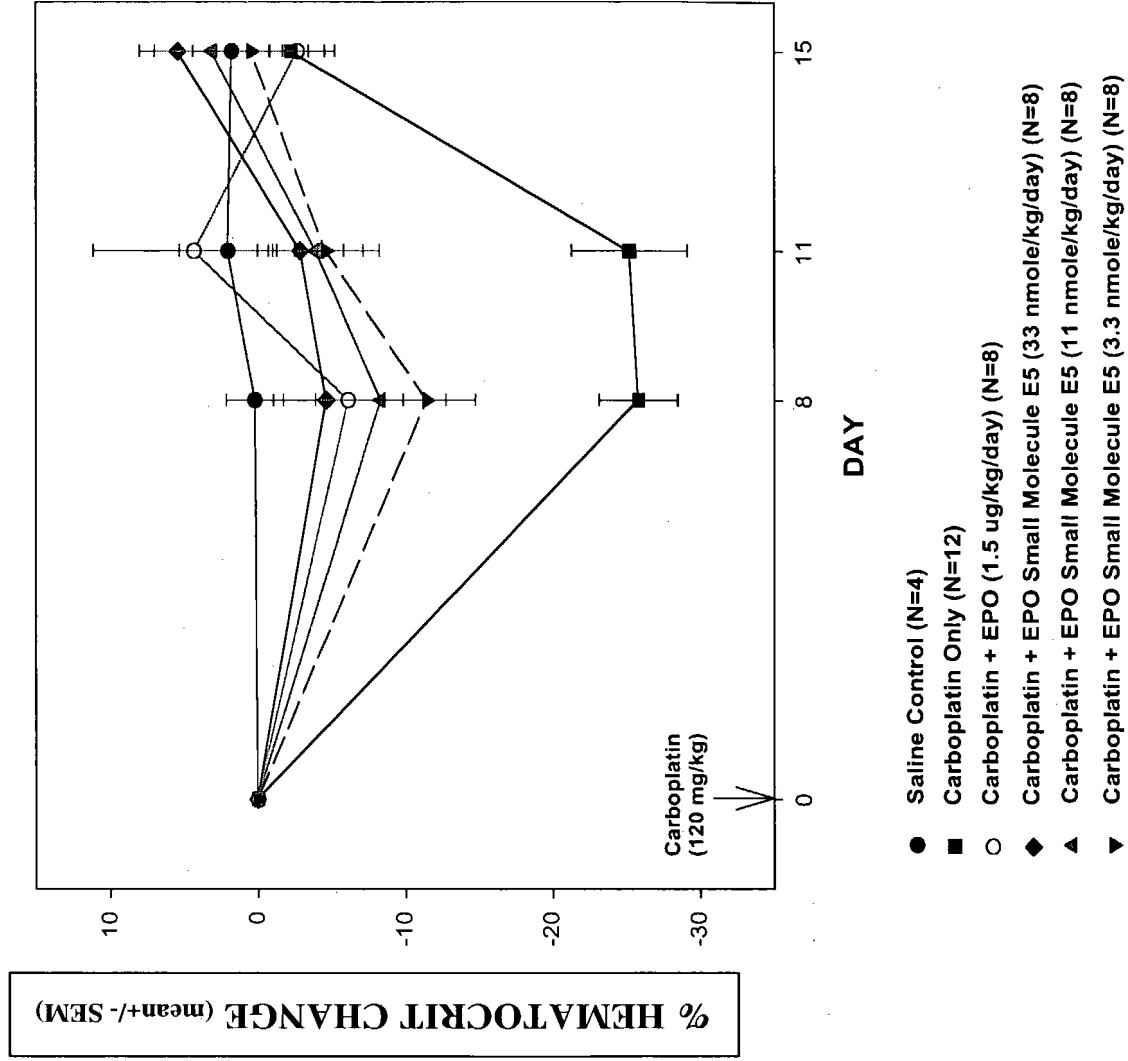
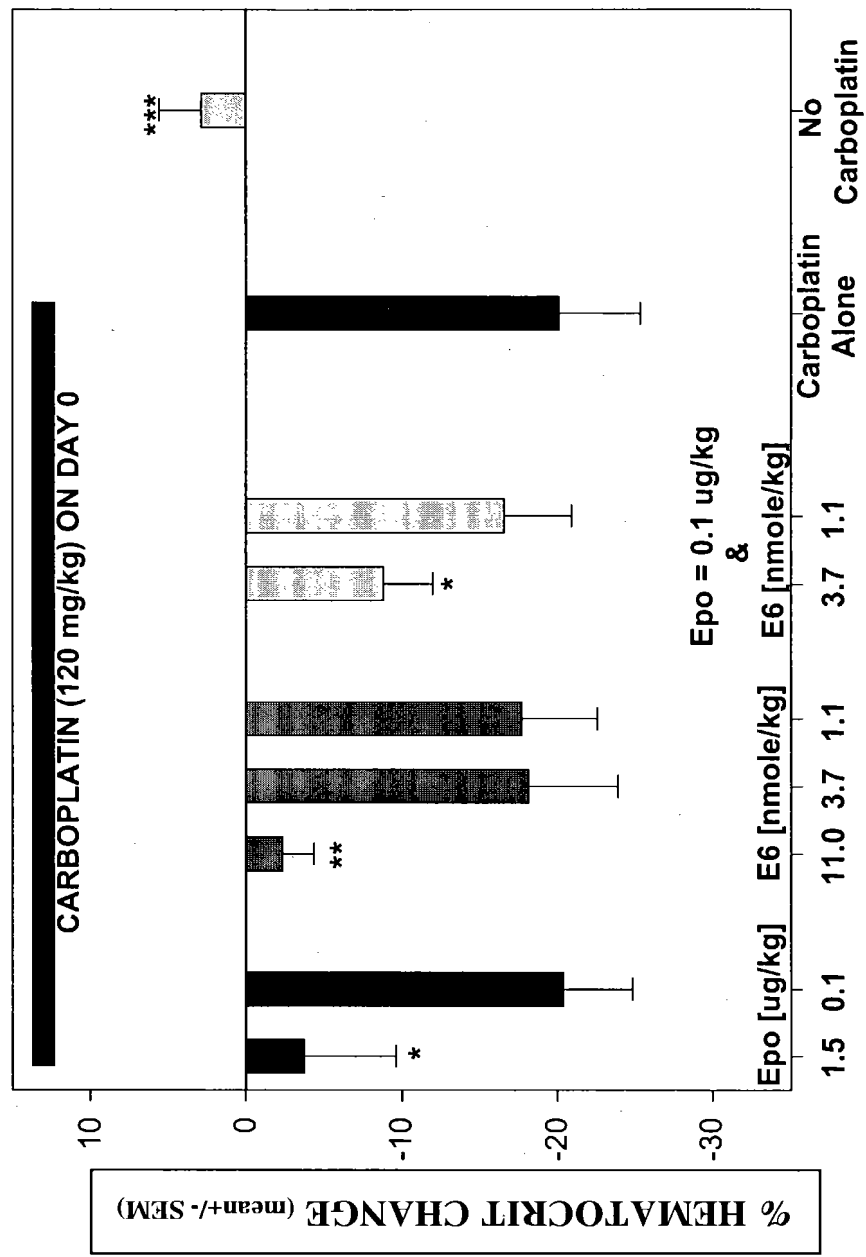


FIGURE 10

SYNERGISTIC
EFFECT ON
CARBOPLATIN-
INDUCED ANEMIA BY
ERYTHROPOIETIN
WITH EPO SMALL
MOLECULE E6 IN 8
WEEK OLD MALE
C57BL MICE
DAY 10



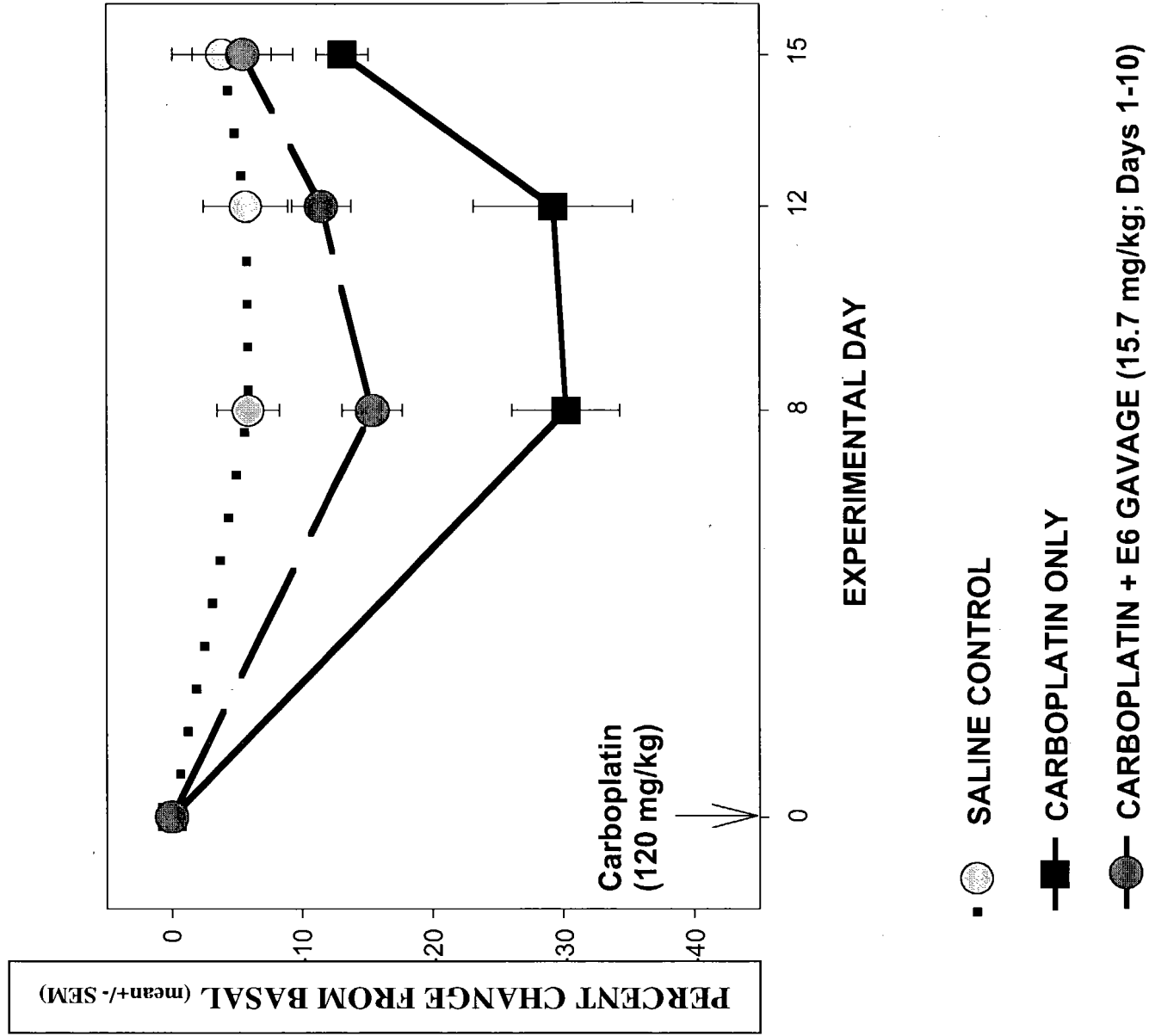
- Carboplatin + Epo
- Carboplatin + Epo Small Molecule E6
- Carboplatin + Epo (0.1 ug/kg) + Epo Small Molecule
- Carboplatin Alone
- No Carboplatin

* p<0.05 Versus Carboplatin Alone
** p<0.01 Versus Carboplatin Alone
*** p<0.005 Versus Carboplatin Alone

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FIGURE 11

**EFFECT OF E6
ADMINISTERED
BY GAVAGE ON
HEMATOCRIT IN 8
WEEK-OLD MALE
C57BL/J MICE**



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FIGURE 12

Effect of EPO -
small molecule
on reticulocyte
levels in normal
animals

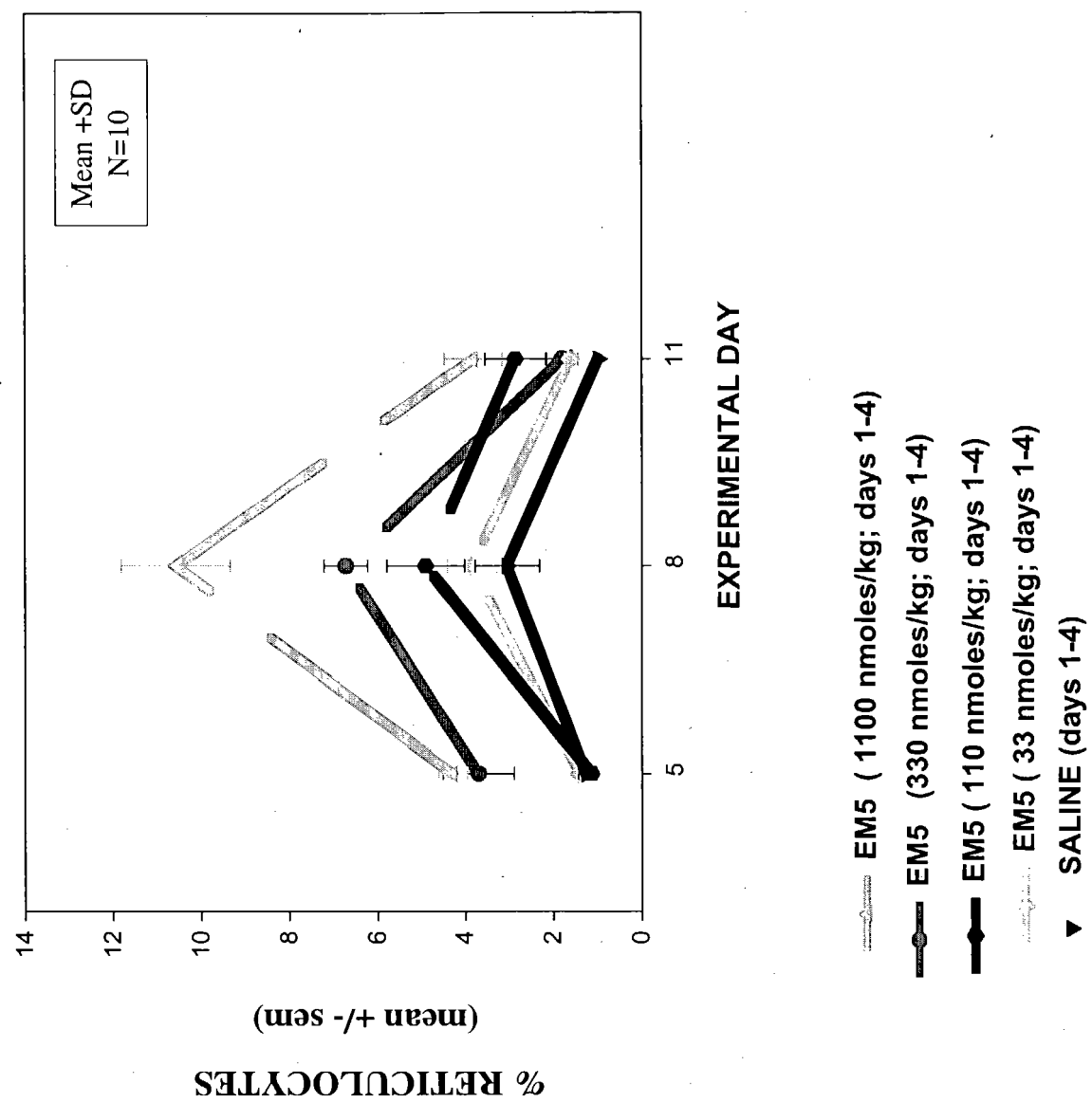
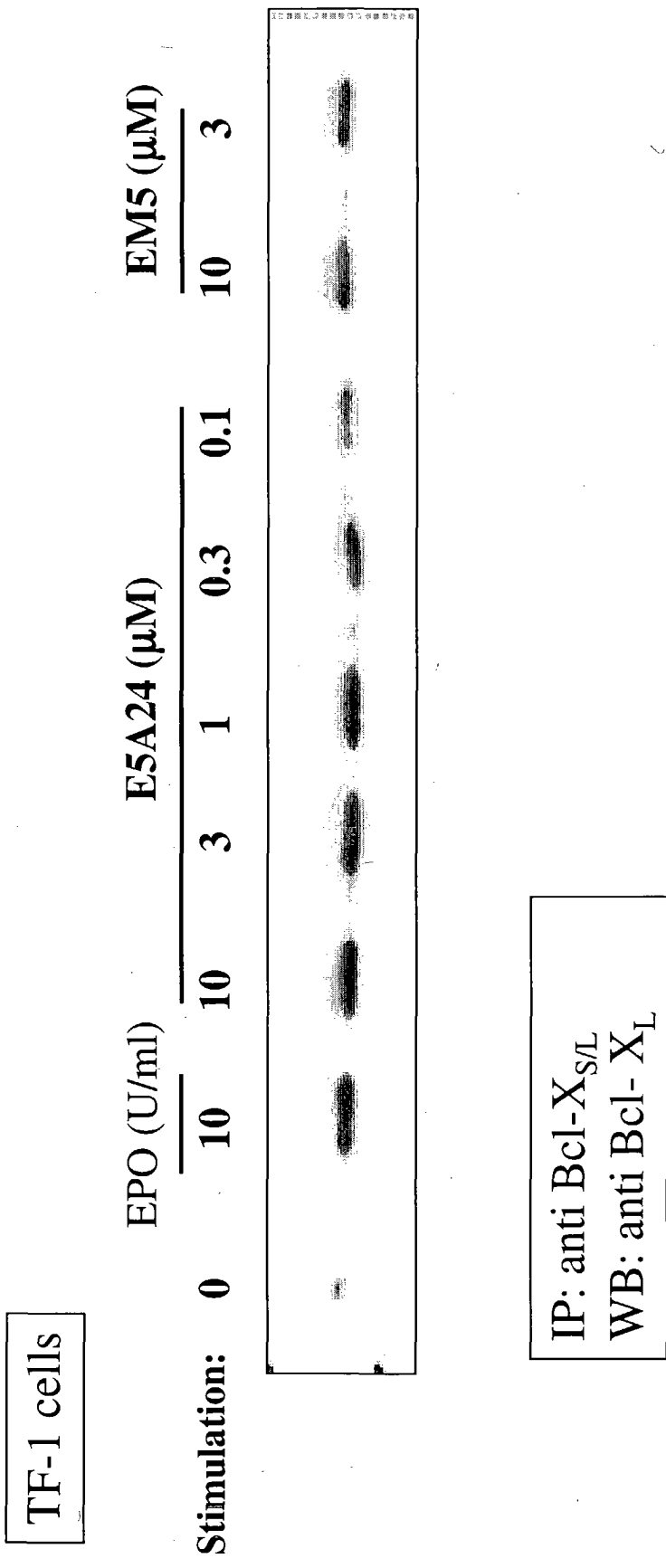


FIGURE 13 --Small molecule activates Bcl-X_L expression



LI/c1

FIGURE 14--Small molecules activate Bcl-X_L
expression



FIGURE 15 --Effect of small molecules on P19 cells

- P-19 is a neural-like embryonal carcinoma cell line that undergoes apoptosis upon withdrawal of serum

Protocol:

- * Treatment with small molecule or EPO 24 hrs prior to serum withdrawal
- * Serum withdrawal for 48 hours + EPO or small molecule

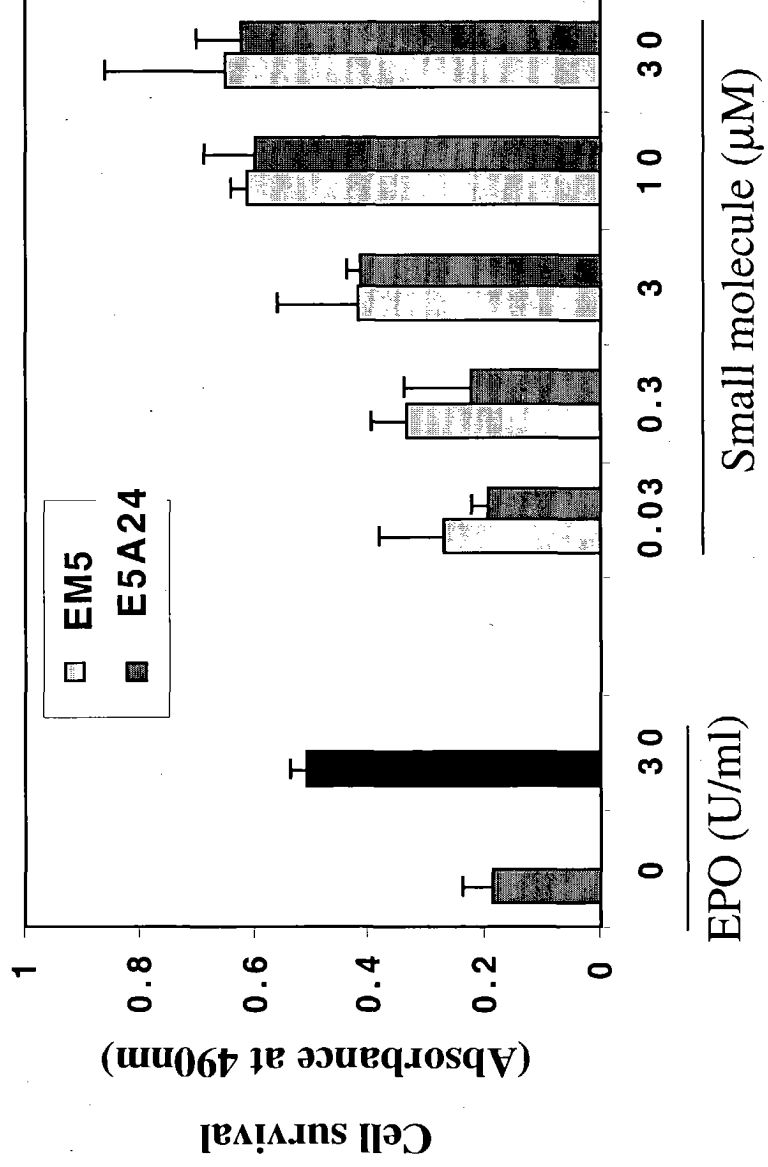
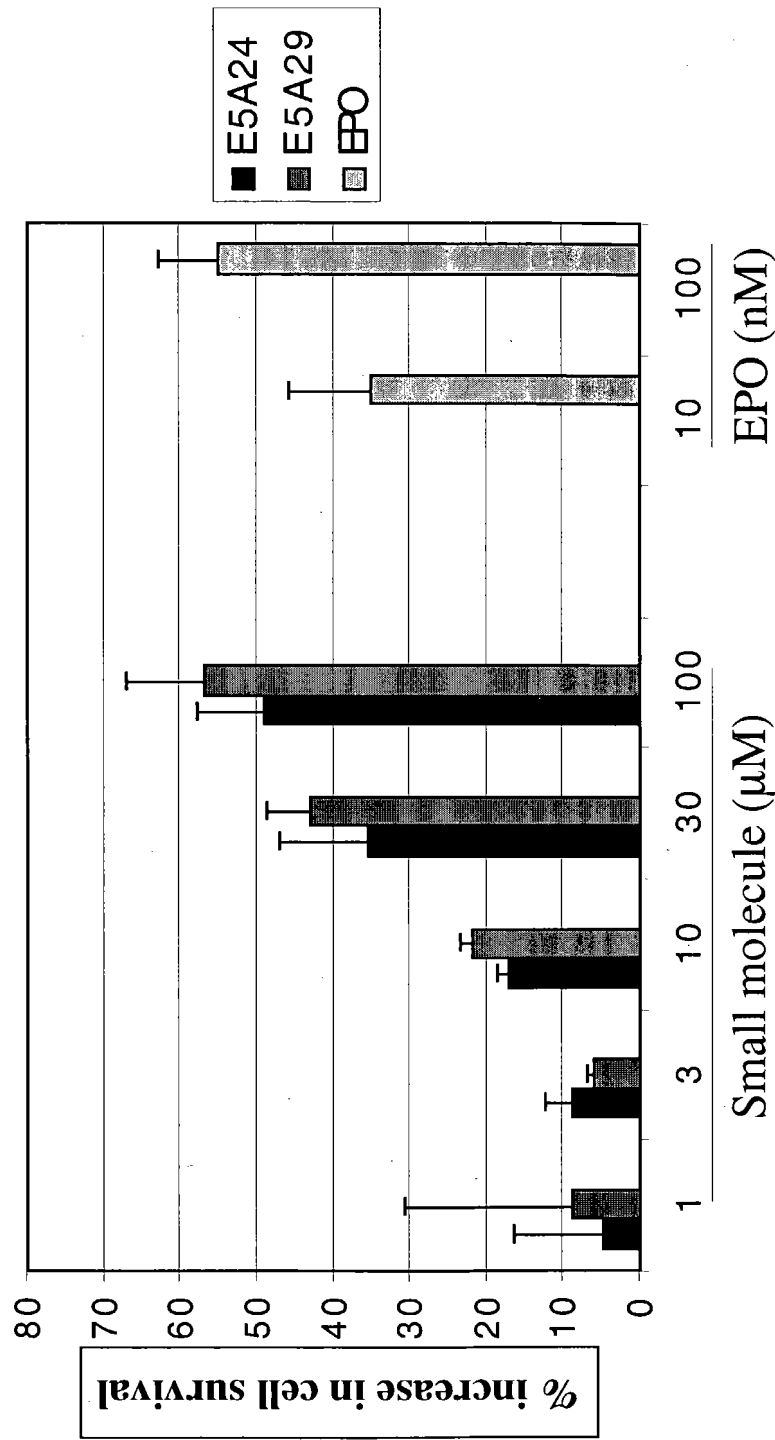


FIGURE 16 --Small molecules prevent neuronal apoptosis
after glutamate challenge



* Cortical Neurons isolated from Embryonic day 18 rats
 * Treatment with small molecule or EPO for 24 hrs
 * Challenge with 300 μM Glutamate for 24 hrs

L1/a1

FIGURE 17 --Characteristics of EPO-like activity small molecules

- > Bind to EPO-R and Activate EPO-R signaling pathway in the presence and absence of hormone
 - Bind to different site than hormone and do not interfere with EPO binding
- > Act on early erythroid progenitors
 - CFU-e/BFU-e formation in fetal liver cells
 - Synergistic effect with EPO in Bone marrow cells (mouse and human)
- > Promote EPO like anti-apoptotic activity
 - Expression of Bcl-X_L protein
 - Increase in neuronal survival during glutamate challenge
- > Restore hematocrit level in *in vivo* animal model, given I.P. and orally
 - Act in synergy with EPO
- > Increase reticulocyte levels in normal animals